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Effects of pH Changes on Phytoplankton Biomass

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A phytoplankton was incubated for a week to determine the effects of pH changes on its biomass growth in nutrient enrichment incubation samples. In this experiment, a general increase in phytoplankton biomass was observed after 24 hours of incubation, with a similar growth pattern in all incubation samples. By comparing with the same pH range (7.0 to 8.0), Pulau Pangkor (PP) incubation samples achieved the peaks earlier compared to Pulau Redang (PR) samples, although they had a higher increment in biomass. Meanwhile, in extreme pH (4.0 and 9.0) incubation samples, the phytoplankton biomass was observed to thrive well. This present study suggests that the phytoplankton community in Pulau Redang and Pulau Pangkor waters is able to survive in a wide range of pH levels, and the change in ocean pH has no vital impacts on the phytoplankton based on the short-term experiment.

Keywords: enrichment incubation experiment; seasonal monsoon; ocean acidification

I. INTRODUCTION

Phytoplankton are microscopic marine algae that consist of a wide taxonomic range, including cyanobacteria, diatoms, dinoflagellates, and coccolithophores (Lindsey & Scott, 2010). They are unicellular and colonial organisms that form the base of aquatic food webs. According to Yuan *et al.* (2014), they convert chlorophyll and electromagnetic radiation from sunlight (wavelength range 400–700 nm) into organic compounds from dissolved components in seawater (carbon, phosphorus, and nitrogen), and produce energy through photosynthesis. They are widely distributed in marine and estuarine environments (Saifullah *et al.,* 2019). Marine phytoplankton are dominated by microalgae known as dinoflagellates and diatoms, even though other algae and cyanobacteria can be present (Dokulil & Teubner, 2000; Leterme *et al.,* 2020). Phytoplankton are the base of aquatic ecosystems that form a fundamental link in the food chain and also act as bait for breeding aquatic creatures such as shrimp, shellfish, and fish (Yuan *et al.*, 2014). Other than that, they are also able to contribute to roughly one-third of the global primary production (Mattei *et al.,* 2018; Käse & Geuer, 2018).

The biomass of phytoplankton is widely used as an indicator of ecosystem productivity and trophic status (Moran *et al.,* 2010; Moran & Scharek, 2015). This has potential due to its small individual size and short life cycle, which allow it to respond to rapid changes in the environment (Yuan *et al.*, 2014). The varieties of phytoplankton distribution and abundance are possibly caused by the differences in local environmental conditions. As mentioned by Cunha and Calijuri (2013) and Mohamed and Amil (2015), the species composition, succession, and abundance of phytoplankton were determined by several limiting factors, such as nutrients, light, and temperature.

Phytoplankton plays a significant role in balancing the carbon cycle. They are the key mediators of the biological pump that can change environmental conditions and predict future atmospheric concentrations of carbon dioxide (Basu & Mackey, 2018). Their abilities to consume carbon dioxide are on a scale equivalent to those of forests and other land plants, where the process of inorganic carbon uptake from the atmosphere is later converted into organic carbon and stored in their body cells. When the phytoplankton die, some of this carbon is carried to the deep oceans, and some is transferred to different layers in the water column.

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Phytoplankton exhibit seasonality, where some species are present throughout the year, whereas others might only be found at certain periods of the year (Stelling *et al.,* 2023). In general, these species are seasonal, and they may last only a week or two annually. Seasonal blooms of phytoplankton are closely linked to monsoon-related changes in winds (Lindsey & Scott, 2010). There are two monsoon wind seasons that are faced by Malaysia's climate. Weaker southwest monsoon wind occurs from April to August that drives a northward coastal jet off Vietnam, whereas stronger northeast monsoon winds occur from November to March, which causes a southward coastal jet in the South China Sea (Chu *et al*., 1999).

The strong physical wind that occurs along the east coast of Peninsular Malaysia may reach more than 30 knots during the monsoon (Mohamed & Amil, 2015). It influences a plankton community structure (Landry *et al*., 1998) and increases the supply of nutrients in the euphotic zone (seawater surface) through the processes of upwelling and vertical mixing (Akhir *et al*., 2012; Mohamed & Amil, 2015). Therefore, the nutrient-rich water increases the productivity of the phytoplankton.

Recently, most studies have focused on nutrient availability, as it is one of the most important factors for phytoplankton growth (Wasmund & Siegel, 2008). According to Dogiparti *et al.* (2013), nitrogen in the form of ammonium (NH_4^+), nitrite (NO_2^-) and phosphate (PO_4^-) are the major limiting nutrients for phytoplankton. Kilhan and Hecky (1988) found that the limiting nutrient for both marine and estuarine phytoplankton is nitrogen, whereas the limiting nutrient for freshwater phytoplankton is phosphorus.

The other factor that can influence primary production is the seawater pH. The pH parameter is a measurement of the hydrogen ion (H^+) concentration of a solution; a high concentration of H^+ indicates a low pH, and vice versa. Generally, it is assumed that the pH in marine systems varies little around a typical surface value of 8.2 (Tarldsvik & Myklestad, 2000). At this average pH level, 90% of total carbon dioxide (CO_2) is found as molecular bicarbonate (HCO_3^-), only 1% as CO_2, and the rest as carbonate ions (CO_3^{2-}) (Steeman Nielsen, 1975). There are a few factors affecting pH sensitivity due to changes in the environment, such as temperature, salinity, carbon dioxide, partial pressure, and total alkalinity (Omstedt *et al*., 2010). Besides, pH is a pivotal factor in the variability of each species of phytoplankton. However, inadequate study of pH on the growth rate and ecology of marine phytoplankton due to the assumption of "a constant seawater pH" causes it not to be considered as one of the possible factors (Hinga, 2002).

Studies have demonstrated that slight changes in pH can significantly affect marine systems despite the strong buffering capacity of the carbonate system in seawater (Pegler & Kempe, 1988; Hurd *et al*., 2009). Chen and Durbin (1994) stated that a variation in pH affects the growth of phytoplankton in a number of ways and may be an important factor in regulating its abundance and distribution. Additional removals of inorganic carbon can either lower or raise the pH level. The changes in pH can alter the distribution of carbon bioavailability indirectly while also altering the availability of trace metals and essential nutrients that potentially cause direct physiological effects on the phytoplankton at extreme pH levels (Chen & Durbin, 1994). Most of the experimental data used monoculture to observe the effects of pH in a small range on phytoplankton, and some showed quite a similar result based on the growth rates (Chen & Durbin, 1994; Hinga, 2002; Shi *et al*., 2009; Berge *et al*., 2010). Meanwhile, few studies used natural phytoplankton communities, which resulted in species composition alteration in response to lowered pH (Pedersen & Hansen, 2003; Feng *et al*., 2009).

Here, we have focused primarily on the effects of ocean acidification phenomena on a phytoplankton community. In this study, the aim is to determine the effects of pH changes (4.0–9.0) on the growth of phytoplankton biomass in the nutrient enrichment incubation samples collected from Pulau Redang and Pulau Pangkor.

II. MATERIALS AND METHOD

A. *Seawater Sampling*

Samplings were carried out on October 5, 2019 at Pulau Redang, Terengganu, and on October 31, 2019 at Pulau Pangkor, Perak (Figure 1). Seawater samples of 10 L at the surface layer (3 m depth) were collected by using a van Dorn water sampler at a few selected sampling stations for the enriched incubation experiment. In-situ parameters for each sample were recorded using YSI Pro Plus.

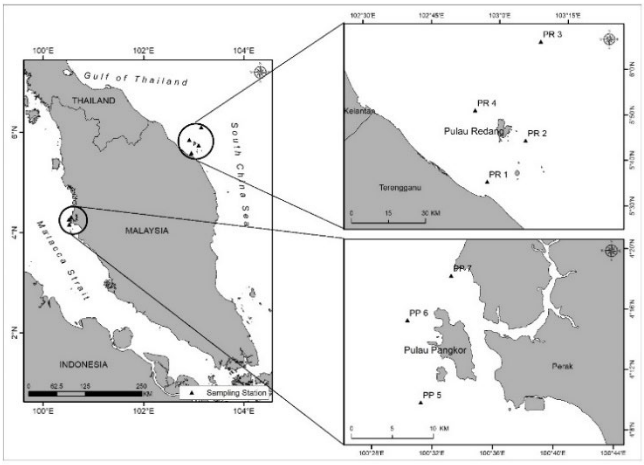

Figure 1. Map showing the location of the selected sampling stations at Pulau Redang (PR) (top) and Pulau Pangkor (PP) (bottom).

B. Nutrients Preparation

A combination of nutrients was added to the incubation samples to induce phytoplankton biomass growth. The main nutrients needed by phytoplankton are carbon, nitrogen, and phosphorus. Glucose ($C_6H_{12}O_6$) was used as the source of C, ammonia chloride (NH_4Cl) as a source of N, and di-sodium hydrogen phosphate (Na_2HPO_4) as a source of P. The preparation of the nutrient solutions was done as per suggested by Mohamed and Amil (2015).

C. pH Incubation Analysis

To gain a better understanding of the effects of pH changes on phytoplankton biomass, we conducted two experiments with different pH ranges that mimic the natural seawater and extreme conditions. 10 L of unfiltered surface seawater samples were collected from Pulau Redang (PR) and Pulau Pangkor (PP) to serve as a representation of the natural phytoplankton community. Collected samples were divided into a few subsamples in incubation bottles with a volume of 250 mL. Seawater samples collected from Pulau Redang were subsampled to four different pHs (7.0, 7.5, 8.0, and 8.5), whereas samples collected from Pulau Pangkor were subsampled to six different pHs (4.0, 5.0, 6.0, 7.0, 8.0, and 9.0). Hydrochloric acid (HCl) and ammonia solution (NH_4OH) were used to adjust the pH of each incubation sample. All the incubation samples were incubated in an incubator at the ambient ocean temperature of 28 °C with an exposure to ultraviolet (UV) rays. The phytoplankton's biomass in all samples was monitored every day for up to a week by using a spectrophotometer, to detect the concentrations of chlorophyll-*a*. The growth of phytoplankton biomass in each sample was measured at a wavelength between 550 nm and 600 nm (Mohamed & Amil, 2015).

D. Calculations and Statistics

Exponential growth rates in μ (day^{-1}) were calculated as:

$$\mu = ln\ (Xt_2 - Xt_1)/t_2 - t_1 \qquad (1)$$

where Xt_2 and Xt_1 is the biomass growth at the end (t_2) and start (t_1) with a sampled interval of 24 hours. All statistical analysis were done using IBM SPSS Statistic version 25. Analysis of variance (ANOVA) was used to test for differences in growth rates. A significance level of <0.05 was chosen.

III. RESULT AND DISCUSSION

A. Phytoplankton Biomass Growth Pattern

A natural marine phytoplankton biomass was quantified in bulk by using a spectrophotometer to detect the adsorption of chlorophyll-a. Nutrients were added to promote the rapid growth of phytoplankton and to observe the effects of pH changes on the phytoplankton within a brief timeframe. The changes in chlorophyll-*a* concentration are commonly used as a proxy for phytoplankton production and biomass (Olonscheck *et al.*, 2013).

All stations at Pulau Redang showed the same pattern of growth, whereby an increase in biomass was observed after 24 hours of incubation. The phytoplankton achieved the highest peak at 48 hours except in pH 8.0 incubation (PR 3) (Figure 2). Among these stations, PR 2 and PR 4 showed the highest biomass increase of 64-fold within 24 hours in pH 7.0 and 7.5 incubations. Meanwhile, PR 3 showed the highest biomass increase of 61-fold and 99-fold in pH 8.0 and 8.5 incubations, respectively. After 48 hours of incubation, the biomass in all pH ranges dropped, and the readings were similar to the initials at the end of the experiment.

From the growth pattern in seawater samples from Pulau Pangkor, an increase in biomass was observed after 24 hours of incubation in all incubation samples (Figure 3). However, only in pH 4.0 incubation did the biomass achieve the highest peak at 48 hours, whereas the others (pH 5.0–9.0) achieved the highest peak earlier, which occurred at 24 hours. The highest biomass increases between the pHs occurred in PP 6, whereby an increase of 9-fold (pH 4.0 and 7.0), 11-fold (pH 5.0), 7-fold (pH 6.0), 13-fold (pH 8.0), and 5-fold (pH 9.0) occurred within 24 hours of incubation. After 24 hours, most of the biomass decreased until the end of the experiment.

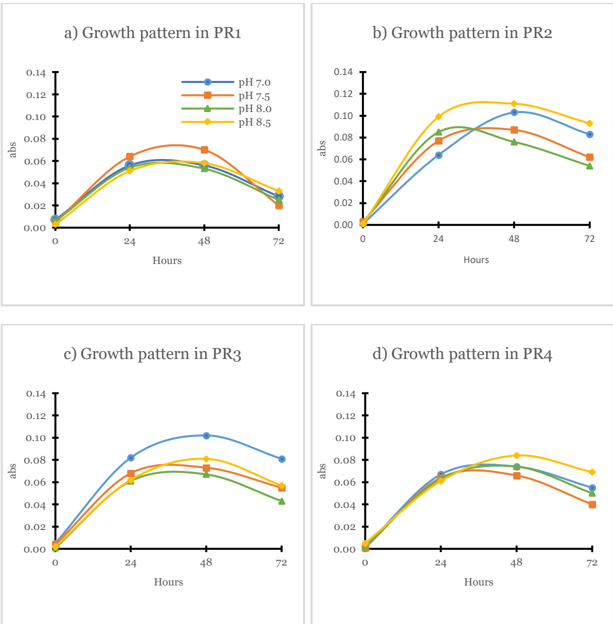

Figure 2. Growth pattern of natural marine phytoplankton community in a) PR 1, b) PR 2, c) PR 3 and d) PR 4 at pH range 7.0 – 8.5.

Samples from Pulau Redang stations' initial readings of chlorophyll-*a* were detected below 0.008 abs, and Pulau Pangkor stations were below 0.04 abs, which showed a 50-fold difference between the two sides. Contrary to the study reported by Lim and Lee (2015), Pulau Redang has a lower amount of biomass compared to other coastal water systems. Contradicting Pulau Pangkor, which serves as one of the main tourist attractions on the west coast of Peninsular Malaysia, the overdevelopment and improper waste disposal systems in coastal zones (Praveena *et al.*, 2012) largely contributed to higher initial biomass readings due to the high input of nutrients.

An increase in biomass within 24 hours from Pulau Redang incubation samples was observed to be higher than that from Pulau Pangkor incubation samples at the same pH (7.0 and 8.0) (Table 1). It suggested that the seawater at Pulau Redang has an insufficient amount of nutrients (Lim & Lee, 2015), which hindered the growth of phytoplankton as the phytoplankton poised to grow with the addition of nutrients in the experiment (Mohamed & Amil, 2015).

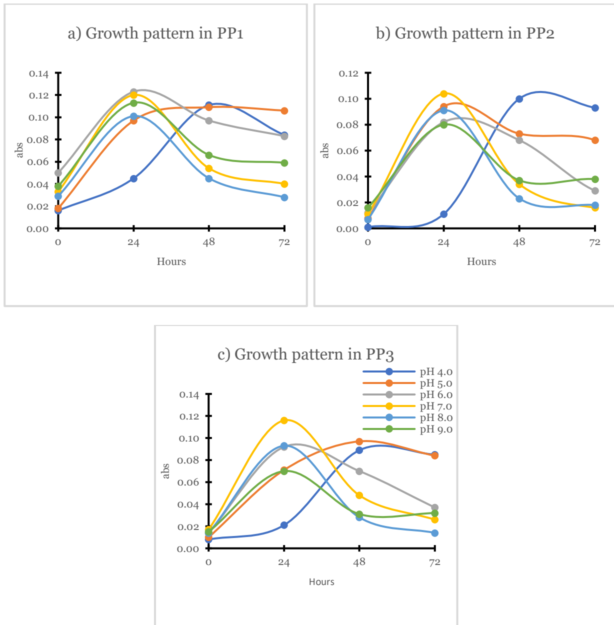

Figure 3. Growth pattern of natural marine phytoplankton community in a) PP 1, b) PP 2 and c) PP 3 at pH range 4.0 – 9.0.

Table 1. Differences in phytoplankton biomass in pH 7.0 and 8.0 incubations between Pulau Redang (PR) and Pulau Pangkor (PP).

pH	Pulau Redang (PR) abs		Pulau Redang (PR) Inc.	Pulau Pangkor (PP) abs		Pulau Pangkor (PP) Inc.	Ratio (PR/PP)
	0H	24H		0H	24H		
7.0	0.001	0.064	64 x	0.011	0.104	9 x	7.1
8.0	0.001	0.061	61 x	0.007	0.091	13 x	4.7

There was no apparent lag phase observed in all pH ranges (except for pH 4.0 incubation) during the incubation period (Figures 3 and 4). In pH 4.0 incubation, the lag phase was recorded for a short period only (<24 hours). No apparent lag phase was recorded at the beginning of the experiment, this might be due to the fast adaptation of phytoplankton to new sources of nutrients, light exposure intensity, or temperature shock (Irwin *et al.*, 2015; Mohamed & Amil, 2015).

Meanwhile, in pH 4.0 incubation, the phytoplankton was still adapting to the new conditions (nutrient addition). After 24 hours, the phytoplankton biomass on both sides began decreasing until the end of the incubation period. The decrement in phytoplankton biomass might have been caused by the insufficient addition of nutrients in early incubation, which could have hindered their ability to continually grow, and the grazing effects of zooplankton within the samples might have contributed as well (Pedersan & Hansen, 2003). Some species in the phytoplankton community also have the tendency to disappear completely throughout the incubation period (Pedersan & Hansen, 2003).

The focus of the phytoplankton group was diatoms and dinoflagellates, as they were the most dominant classes in most of the previous experiments conducted in the region (Li *et al.,* 2011; Lim & Lee, 2015; Mohamed & Amil, 2015; Zhou *et al.,* 2017). According to Margalef (1978), diatoms are usually dominant in areas with rich nutrients, whereas dinoflagellates are often found in areas with low nutrients. This present study has deduced that diatoms dominate the composition of the phytoplankton in Pulau Redang, as reported by Lim and Lee (2015). It is dominated by *Chaetoceros* sp.. According to Wu and Chou (2003), such predominance could occur as it employs the r-strategy because of its small size and ability to respond to nutrient-limiting conditions.

Despite the low nutrients input and low phytoplankton biomass in Pulau Redang (Lim & Lee, 2015), results from this study suggest that the phytoplankton community of the site of interest is able to survive within the pH range of 7.0–8.5. However, a lower pH (<pH 7.0) could interrupt the phytoplankton growth, raising concerns about the sensitivity of phytoplankton in Pulau Redang to environmental changes. This is due to the fact that Pulau Redang is one of the marine parks in Malaysia where the least anthropogenic activities lead to drastic changes, which is different compared to Pulau Pangkor.

B. *Phytoplankton Growth Rate*

Based on statistical analysis (Figure 4 and Figure 5), the growth rates in all pH ranges were similar, as there was no significant difference. From the overall observation, the growth rate in Pulau Redang incubation samples reached its maximum within 24 hours in the pH range of 7.0–8.5. On the next 24-hour cycle (48 hours), it decreased drastically and continued to be in the negative range at 72 hours (Figure 5). However, only pH 8.0 from all stations was observed to maintain a positive growth rate for up to 48 hours of incubation compared to others. This was in contrast to the results from Pulau Pangkor incubation samples, where the positive growth rate only occurred within 24 hours.

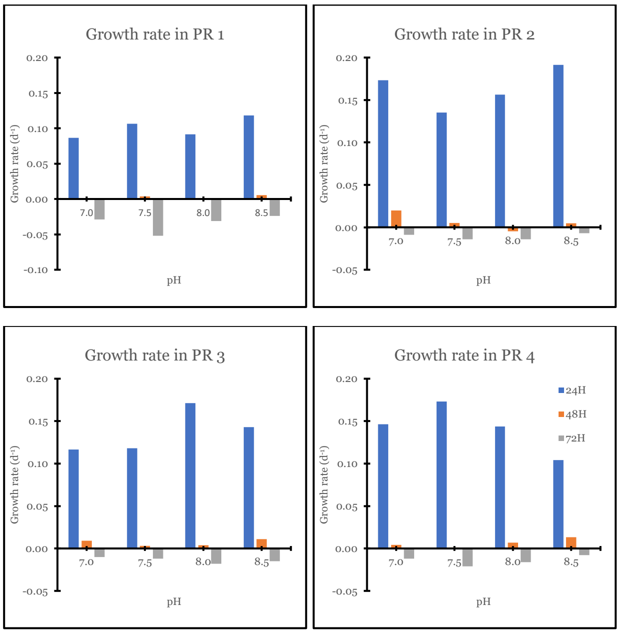

Figure 4. The growth rate of the natural marine phytoplankton community in Pulau Redang within 72 hours of incubation.

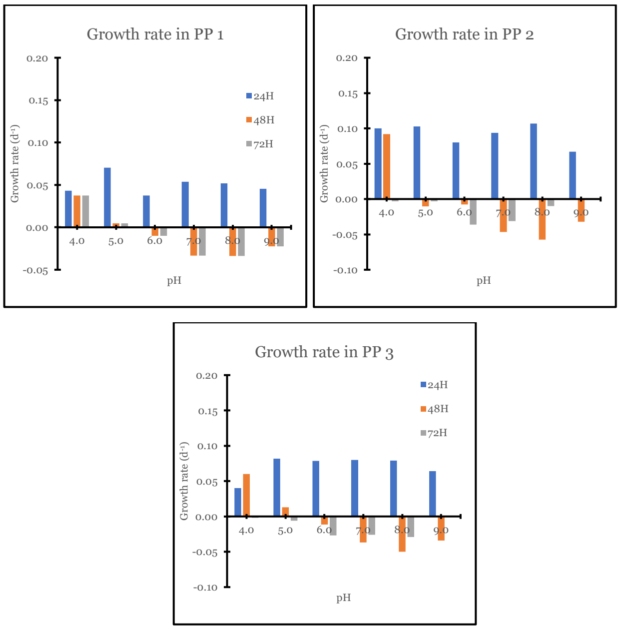

Figure 5. The growth rate of the natural marine phytoplankton community in Pulau Pangkor within 72 hours of incubation.

However, the results from both sites showed a similar range as previously published phytoplankton growth rates (Table 2), with the exception of no specific species in our experiment. The differences in species composition that thrive at different pHs were based on their tolerance to pH changes and their ability to maintain a favourable intracellular pH (Berge *et al.,* 2010; Labare *et al.,* 2010). According to Pedersen and Hansen (2003), the effects of the pH on the phytoplankton in nature are highly dependent on the duration of exposure and the extent to which species can be reintroduced.

Table 2. Marine phytoplankton group, species and pH range for maximum exponential growth based on previous studies. The pH range for maximum exponential growth is defined as the pH over which growth rates were at their maximum levels.

Phytoplankton groups	**Species**	**pH range for max. exponential growth rate**	**Sources**
	Prorocentrum minimum	7.3 - 8.9	Hansen (2002)
		7.0 - 8.4	Berge *et al.* (2010)
	Prorocentrum micans	7.0 - 8.4	
Dinoflagellates	*Heterocapsa triquetra*	6.9 - 8.7	
	Heterocapsa triquetra	7.6 - 9.1	Hansen (2002)
	Ceratium lineatum	7.4 - 8.5	
	Karlodinium veneficum	7.0 - 8.3	Berge *et al.* (2010)
Diatoms	*Thallasiosira oceanica*	7.1 - 8.9	Chen and Durbin (1994)
	Thallasiosira pseudonana	7.1 - 8.9	
	Coscinodiscus granii	7.1 - 8.4	Berge *et al.* (2010)

Meanwhile, at the extreme condition tested (pH 9.0) in this experiment, the phytoplankton in Pulau Pangkor incubation samples showed a similar pattern of growth rate to other pH incubations. According to Pedersen and Hansen (2003), some pH-tolerant species were able to thrive well in pH 9.0 incubations. A well-grown phytoplankton biomass at a high pH (9.0) could result in low grazing that poses a high mortality rate for copepod and protozooplankton communities (Pedersen & Hansen, 2003). Besides, it was hypothesised that exposing phytoplankton to high pH conditions would lead to a substantial reduction in species richness and result in long-lasting changes in their composition (Pedersen & Hansen, 2003; Flynn *et al.*, 2015).

At the same time, it was believed that when phytoplankton are exposed to high pH conditions, their species richness will also significantly decrease for extended periods of time (Pedersen & Hansen, 2003).

Although there are yet to be relevant studies conducted with a pH below 6.0, this study proved that the natural community of phytoplankton was able to survive in these extremely acidic conditions (< pH 7.0). They had shown a similar pattern of growth rates with others, but only at pH 4.0. It showed a different pattern, based on Figure 6, where a positive growth rate was observed that extended until 48 hours of incubation. Berge *et al.* (2010) found that the growth rate of the cryptophyte *Teleaulax amphioxeia* was also unaffected, even at a lowered pH of ~6.1. This proposes that the phytoplankton growth rates in general from both study sites are tolerant of ecological changes in pH over a short-term period. On the other hand, picophytoplankton also have a high survivability rate upon the increment in acidification, and showed no significant affliction, especially in the abundance of total bacteria (Allgaier *et al.,* 2008; Newbold *et al.,* 2012). Besides, there is also a possibility of community composition shifting occurring at pH 4.0 (Figure 6). According to Dutkiewicz *et al.* (2015), picophytoplankton were able to survive in extremely acidic conditions due to their higher growth rate response (GRR) based on the model marine ecosystem taken from pre-industrial conditions until 2100.

It is predicted that by 2100, the ocean pH levels will reduce until they reach pH 7.67, which is roughly five times the amount of acidification in the past decades (Kennedy, 2010). Based on this present study, the short-term incubation showed that the phytoplankton biomass growth in Pulau Redang and Pulau Pangkor might not be affected by ocean acidification in the near future. It has been proven that the phytoplankton from Pulau Redang are able to thrive in conditions as low as pH 7.0 (Figure 3), whereas those from Pulau Pangkor are able to thrive in conditions as low as pH 4.0 (Figure 4). Even though there was no phytoplankton composition recorded, this study suggests that there might be changes in the community composition at low pH, especially at pH 4.0, based on the trend of growth rates shown in Figure 5.

IV. CONCLUSION

In summary, this short-term experiment with a fixed pH exposed to the phytoplankton community showed positive effects on biomass growth from low to high pH levels. Although there are monsoonal seasons in Malaysia, the pH range (7-8) between pre- and post-monsoon has been proven to not affect the phytoplankton community in Pulau Redang and Pulau Pangkor.

Considering the ongoing ocean acidification, if the pH is continually decreasing (pH<7.0), changes in community composition due to different coping mechanisms, such as the CO_2 concentrating mechanism (CCM), of each species present might occur. The lack of understanding on this matter explains the need to filter the seawater to lower the grazing effects of microzooplankton on phytoplankton and to identify the composition of the natural phytoplankton community, which is to improve current knowledge. Hence, it is hoped that future studies will be able to interpret the possibilities of certain phytoplankton groups that might be afflicted with pH variation.

V. ACKNOWLEDGEMENT

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